

On the infraspecific variability and taxonomic position of *Entoloma zuccherellii*

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Abstract — A recent find of the enigmatic and extremely rare fungus *Entoloma zuccherellii* in the Czech Republic has given more insight in the infraspecific variation of that species. A detailed description of this find and comparison with Italian and Spanish finds are provided. The taxonomic and phylogenetic position of *E. zuccherellii* is discussed.

Key words — *Entolomataceae*, *Rhodocybe*, Czech Republic, spores

Introduction

In 2007 and 2008, the first author carried out a mycological research of Central Bohemia, a region around Prague, Czech Republic (Holec 2009). In 2007, special attention was focused on Kokořínsko Protected Landscape Area, a sandstone region 40 km NNE of Prague. In the Kokořínský důl Nature Reserve, an interesting lignicolous fungus resembling a small *Entoloma* species with a bluish-brown stipe was found. The first author was unable to identify it using the newest *Entoloma* monograph (Noordeloos 2004). A revision done by the second author showed that it was conspecific with *Rhodocybe zuccherellii* (Noordeloos & Hausknecht 2000), recently transferred to the genus *Entoloma* (Co-David et al. 2009). Records of this species are rare, and it was known previously only from the type locality in Italy and a second collection from Spain (Vila & Caballero 2009). Therefore the Czech find is published here in detail.

Material and methods

The first author microscopically examined material mounted in a 5% KOH solution using an Olympus BH-2 microscope, except for pileus and stipe

cuticle pigments, which were observed in pure water. Spore measurements were determined from 20 randomly selected mature, fully developed spores. Microcharacters were drawn at a magnification of $1250\times$ using a drawing tube. Descriptive terminology follows Bas et al. (1988). Colour terms are English translations of the original field description written in Czech. For colour codes see Kornerup & Wanscher (1981). The collection studied is kept in the herbarium PRM (National Museum, Mycological Department, Prague, Czech Republic). Abbreviations: L = number of lamellae reaching up to the stipe, l = number of lamellulae between each pair of two lamellae, Q = quotient of length and width of the spores, Qav = mean value of Q in the collection studied.

Taxonomy

Entoloma zuccherellii (Noordel. & Hauskn.) Co-David & Noordel.,

Persoonia 23: 175, 2009.

PLATES 1–3

= *Rhodocybe zuccherellii* Noordel. & Hauskn., Bollettino del

Gruppo Micologico G. Bresadola, n.s. 43(3): 29, 2000.

MACROCHARACTERS (based on 3 basidiocarps found; 1 young, 2 mature) — **PILEUS** 4–10 mm, hemispherical with inflexed margin and flattened upper part, mat, slightly hygrophanous, margin indistinctly translucently striate, whole surface scarcely and finely white fibrillose-pruinose, dark brown when young and moist (5F4-6), then dark brown at centre and brown (5E5-6) to ochre-brown (5D6-7) towards margin, margin remaining dark brown when moist; **LAMELLAE** sparse, L = 13–20, l = 1–3, segmentiform, adnate when young, then emarginate, greyish brown-beige (4C3-4) when young, then pale yellowish beige (3A-B3), with concolorous, eroded edge; **STIPE** 7–15 $\times$ 1.5–2.5 mm, cylindrical or slightly broadened towards base, ground colour dark grey-brown (6F2-3) to grey-brown (6E3-4) with a slight steel blue tinge, whitish pruinose when young, then whitish fibrillose to finely fibrillose-scaly at apex, base whitish tomentose; **TASTE** and **SMELL** not recorded.

MICROCHARACTERS — **BASIDIOSPORES** (5.6)6.0–7.6(8.0) $\times$ (5.2)5.6–6.4(6.8) μm , average size 6.9 $\times$ 6.0 μm , Q = 1.06–1.29, Qav = 1.16, variable in size and shape, general shape subglobose to almost globose, rarely broadly ellipsoid, many-angled when fully mature, angles indistinct, usually with one big oil droplet, wall slightly thickened, large number of immature or poorly developed spores present (without angles, of deviating shape, without content, such spores were not measured); **BASIDIA** 27–32 $\times$ 7.0–9.5 μm , larger on lamellae edge, up to 37 $\times$ 10 μm , 4-spored, narrowly clavate to clavate, with slight median constriction, content granular; **BASIDIOLES** 16–27 $\times$ 7–8 μm , narrowly clavate to clavate, hyaline; **LAMELLAE EDGE** fertile, rarely with protruding clavate cells which are slightly larger than basidia, about 40 $\times$ 9–15 μm , with granular



PLATE 1. *Entoloma zuccherellii*, Czech Republic, Kokořinský důl Nat. Reserve (PRM 909361).
Photo J. Holec. Colour photo: <http://www.nm.cz/english/departments/mycology-gallery.php>

content; LAMELLAR TRAMA regular, cells long and cylindrical or shorter and slightly inflated, 4–22 μm broad, hyaline, wall with yellow membrane pigment; PILEIPELLIS a cutis of densely arranged parallel hyphae 4–15(19) μm broad, made up of long and cylindrical or shorter and fusiform to barrel-shaped elements (sometimes with a median constriction), terminal elements 12–20 μm broad, barrel-shaped, clavate, rarely with a mucronate projection, the cuticle is pale brown in mass, elements are hyaline („empty“) with pale yellow membrane pigment as well as a rather pale, granulose intracellular pigment, pileocystidia absent; STIPITPELLIS a cutis of densely arranged parallel hyphae 4–16 μm broad, made up of cylindrical to narrowly fusiform elements, with yellow membrane pigment and fine yellow-brown incrustations when observed in pure water, the cuticle is scarcely covered with narrow (5–7 μm) outgrowths or ascending terminal parts of narrower hyphae of the cutis, caulocystidia absent; CLAMP CONNECTIONS absent in all tissues.

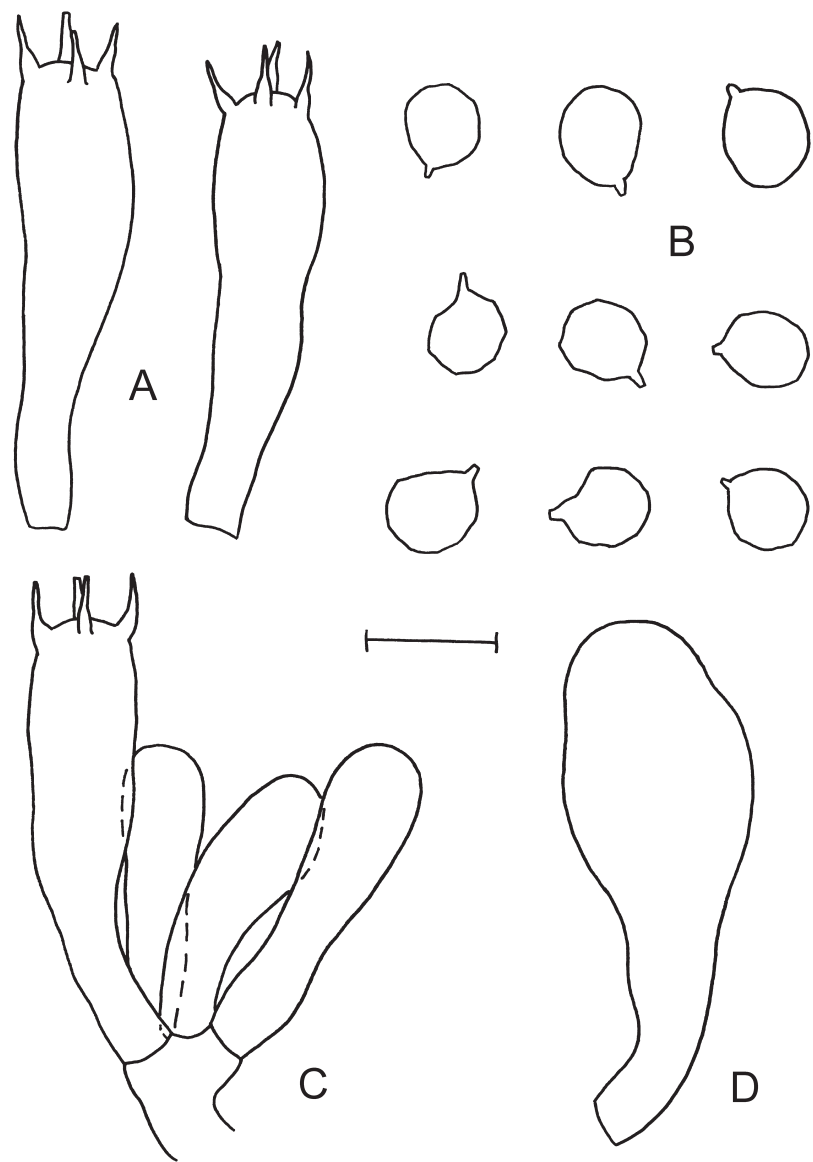


PLATE 2. *Entoloma zuccherellii*, microcharacters (PRM 909361).
A: basidia, B: basidiospores, C: basidium and basidiolae,
D: protruding clavate cell on lamella edge.
Bar = 10 μ m. Drawing by J. Holec.

MATERIAL STUDIED — CZECH REPUBLIC. CENTRAL BOHEMIA, Kokořínsko Protected Landscape Area, ca. 1 km NEE of the village of Kokořín near the town of Mšeno, KOKOŘÍNSKÝ DŮL NATURE RESERVE: trail along Pšovka stream, alt. 250 m, man-influenced mixed forest on E slope among sandstone rocks (*Fagus sylvatica*, *Picea abies*, *Quercus* sp., *Pinus sylvestris*), on decaying coniferous wood (*Picea*?), 19.X.2007 leg. T. Zíbar, det. M.E. Noordeloos (PRM 909361).

Discussion

The Czech collection is in good agreement with the type of *Entoloma zuccherellii* (Noordeloos & Hausknecht 2000) from Italy (Ravenna, Pineta di Classe), both in general appearance and in diagnostic characters such as the lignicolous habit, bluish tinges in the stipe, and (in particular) the small, weakly angled spores. The Czech material deviates slightly in the following characters: lamellae without violet tinge, emarginate at maturity; stipe with less distinct blue or violet tinge; spores slightly larger and slightly more prolonged (in holotype they measure $6.0\text{--}6.5 \times 5.5\text{--}6.0 \mu\text{m}$, $Q = 1.0\text{--}1.2$; $Q_{av} = 1.1$); lamellar edges with scattered protruding clavate cells that do not, however, represent true cheilocystidia; and pileus cuticle of wider hyphae. However, the differences are subtle and seem to demonstrate infraspecific variability.

The collection described from Spain (Vila & Caballero 2009) possesses the most distinct blue-violet tinge among the three collections discussed. The blue-violet tinge is very distinct on the stipe and readily visible even on the pileus surface. However, intraspecific variability with regard to the expression of blue and/or violaceous tinges is a well-known phenomenon within *Entoloma*. Similar species (e.g., *Entoloma vinaceum* (Scop.) Arnolds & Noordel. and the closely related North American species *E. trachyosporum* Largent) have varieties based on the presence or absence of blue-violaceous tinges (Arnolds & Noordeloos 1980, Largent 1994, Noordeloos 2004). The photograph published by Vila & Caballero (2009: fig. 8) shows young and fresh basidiocarps where the blue-violet pigments are very pronounced. In other characters the Spanish collections are very similar to the Czech one (including the presence of cells resembling cheilocystidia).

Concerning the ecology, two records are from the coniferous wood (Italy: *Pinus*, Czech Republic: *Picea*?) and one is from the wood of *Alnus glutinosa* (Spain).

Based on all three collections, *E. zuccherellii* can be characterised as follows: small fungus (pileus up to 17 mm), pileus violet-grey (when fresh and young) or grey- to dark brown with a white pruinose-fibrillose surface, lamellae with bluish or violet tinges when young, stipe tinged blue-violet (stable characters seen in all published collections) with a white fibrillose surface, spores measuring $5.6\text{--}7.0(8.0) \times 5.2\text{--}6.4(6.8) \mu\text{m}$ ($Q = 1.0\text{--}1.3$, $Q_{av} = 1.13$) and globose to subglobose and indistinctly angular, distinct cheilocystidia

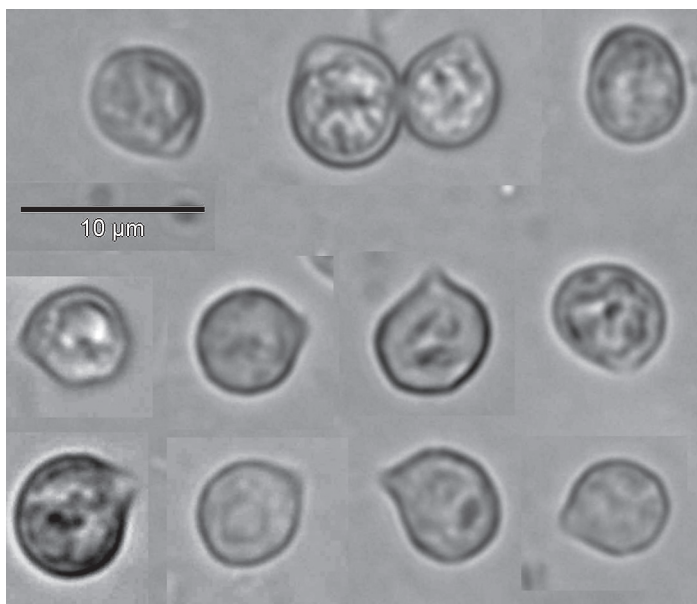


PLATE 3. *Entoloma zuccherellii*, variability of basidiospore shape (PRM 909361).
Bar = 10 µm. Photo by M.E. Noordeloos.

absent, lacking clamp connections, and growing on wood of coniferous and broadleaved trees.

Entoloma pluteisimilis Noordel. & C.E. Hermos., which is microscopically very similar, differs mainly by the lack of blue or violaceous tinges in the basidiocarps. Both *E. zuccherellii* and *E. pluteisimilis* have small, very thin-walled, many-angled spores that resemble those of *Rhodocybe* when observed in the light microscope, which was the main reason that *E. zuccherellii* was published as a new species in *Rhodocybe* (Noordeloos & Hausknecht 2000). SEM studies by Dorien Langeveld (MSc student in Leiden), however, showed that within *Entoloma* a whole gradient can be found from the well-known relatively thick-walled and distinctly angular spores to the thin-walled spores with complete and incomplete facets as well as bumps, similar to those found in true *Rhodocybe* species (Co-David et al. 2009). *Entoloma zuccherellii* and *E. pluteisimilis* both have spores at the bottom end of this range with irregular rugulose surfaces and a few indistinct ribs.

The three-gene molecular phylogeny by Co-David et al. (2009) placed both species in a monophyletic clade within *Entoloma* and distant from *Rhodocybe*, supporting their transfer of *R. zuccherellii* to *Entoloma*. The description of *Entoloma lignicola* Largent, which shares similar small, thin-walled spores and

a lignicolous habit (Largent 1989), suggests that it also belongs in the same clade.

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